Technical problems in centrifugation, as well as variations in materials and goals of the research, led researchers to vary such details as frequency of resolubilizing, length of runs, and speed of centrifugation. For example, due to differences in their initial distribution and other chance factors, some lighter particles sediment along with the heavy particles. In addition, during centrifugation particles will encounter the walls of the centrifugation tube where they may adhere or agglutinate and set up convection currents as they travel down the walls. Small particles may become entrapped and carried down with the larger particles. Empirical explorations revealed that these difficulties could, in part, be overcome by repeated resolubilization and recentrifugation of the sediment. Note again, though, that general expectations regarding the results guided the refinement of the technique.

Are there only four distinct constituents of cells that could be separated by fractionation? There were reasons to suspect more. One of the most powerful reasons was that cytologists already knew of organelles that were not distinguishable in the four basic fractions. For example, de Duve commented, "It may be recalled that the fate in differential centrifugation of such formations as secretory granules, centrosomes, and the Golgi body is entirely unsettled at the present time" (de Duve & Berthet, 1954, p. 250). Shortly after Claude's early work, other researchers developed regimes to isolate additional fractions. Hubert Chantrenne, for example, used the Henroit-Huguenard centrifuge to obtain five fractions whose chemical composition blended into each other. He concluded, "it seems that one can partition the granules in as many groups as one wishes, with no experiments or observations indicating that there exist precise demarcation lines between the different group of particles" (Chantrenne, 1947, p. 445, as quoted in Rheinberger, 1997, p. 64). Novikoff et al. (1953) separated ten fractions and studied distributions of DNA, RNA, nitrogen, and several enzymes in them. As we will see in Chapter 6, procedures for separating additional fractions played a critical role in the 1950s in the discovery of additional cell organelles.

Nonetheless, the four-fraction approach remained dominant. Perhaps the best explanation for this is that these fractions were identified with cell structures for which functions were already suspected. In particular, although it is noteworthy that the scheme provided no place for the Golgi apparatus, some researchers still suspected this structure to be an artifact. Claude in fact collaborated with Palade in a pair of papers in 1949 arguing that it was indeed an artifact. (These papers are discussed in the last section of this chapter.) Until clear evidence as to the function of the Golgi apparatus was developed in the 1960s, it remained possible to doubt its existence and to accept fractionation procedures which did not give it a place.